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GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED MEAL.[1]

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TOXICITY OF COTTONSEED

The term "cottonseed meal" is applied to the ground cake left after the oil is pressed from the seed of cotton (*Gossypium* spp.). For many years it was regarded as a by-product of little value. It is now used extensively as a feed. The annual production of the United States is about 2,000,000 tons, valued at about $53,000,000. While it may be fed profitably to horses, cattle, sheep, etc., in moderate amounts, poisoning and often death occur as a result, especially if the animal has not been gradually accustomed to it. It is generally avoided as a feed for pigs on account of the numerous deaths associated with its use. Dinwiddie (1905) states that hogs show no greater susceptibility than cattle when fed quantities proportional to their body weight. Feeding experiments at the North Carolina Experiment Station have shown that where swine are fed one part of cottonseed meal with three parts of corn meal death generally ensues in from five to seven weeks, although some pigs have been fed for a year or more without fatal results.

In a recent experiment at this Station nine pigs weighing from 75 to 150 pounds were fed in a closed pen on a daily ration of 1 per cent of cottonseed meal and 3 per cent of corn meal, based on their initial body weight. Six died between the thirty-fifth and the fifty-seventh day. The others were alive on the ninetieth day. Roughly, then, 45 per cent of their initial weight in cottonseed meal was fatal to these pigs. All the smaller pigs died.

Withers and Brewster (1913) found that rabbits and guinea pigs would succumb in about 13 days (6 to 22 days) when fed at the rate of 1 per cent of initial body weight daily. Experiments with 22 rabbits showed that, on

[1] This paper is the third in a series of "Studies in Cottonseed Meal Toxicity." Study I, Withers and Ray (1913), is a criticism of Crawford's pyrophosphoric-acid hypothesis; Study II, Withers and Brewster (1913), suggests iron salts as an antidote.

[2] For their cooperation with us in this investigation, we desire to thank Dr. G. A. Roberts and Dr. W. B. Smith, of the Veterinary Department, and Dr. B. F. Kaupp, Pathologist, of the Poultry Department, North Carolina Experiment Station.

an average, 8.3 per cent of initial body weight was sufficient to cause death. These authors make the following statement in regard to these tests:

> As a rule the rabbits ate the meal well during the first few days and made gains in weight. But towards the end they began to refuse the meal in whole or in part and soon thereafter died.

There have been numerous suggestions as to the cause of poisoning and death from the feeding of cottonseed meal. These are summarized in the Experiment Station Record (1910, p. 501) as follows:

> It has been variously ascribed to the lint, the oil, the high protein content, to a toxalbumin or toxic alkaloid, to cholin and betain, to resin present in the meal, and to decomposition products.

Pathogenic organisms and certain fungi have also been suggested.

Friemann (1909), a veterinarian, obtained from the alcoholic extract of cottonseed meal which had caused sickness in cattle a base the platinum salt of which contained 28.75 per cent of platinum. The free base had a paralytic action on exposed frogs' hearts similar to muscarin. He concluded that the toxicity was to be referred to ptomains which result from the nitrogen-containing components of the lecithin, and that unsaturated fatty acids probably contributed to the total action of the meal.

Crawford (1910) concluded that "the chief poisonous principle in certain cottonseed meals is a salt of pyrophosphoric acid." This conclusion is discussed later in this article.

Withers and Ray (1913b) found that the toxicity of cottonseed meal could be destroyed by boiling it with alcoholic caustic soda. This was the only solvent of a large number used which removed or appreciably affected the toxic principle. A noteworthy fact is that the neutralized and evaporated extract was shown to be nontoxic.

Withers and Brewster (1913) found that if a solution of iron and ammonium citrate was fed with cottonseed meal rabbits did not die during a period about seven times as long as the feeding period when iron salts were omitted. Furthermore, rabbits made sick on the meal recovered when the iron solution was supplied with the meal.

PREPARATION OF GOSSYPOL

Our recent experiments have led us to believe that gossypol is the toxic substance of cottonseed.

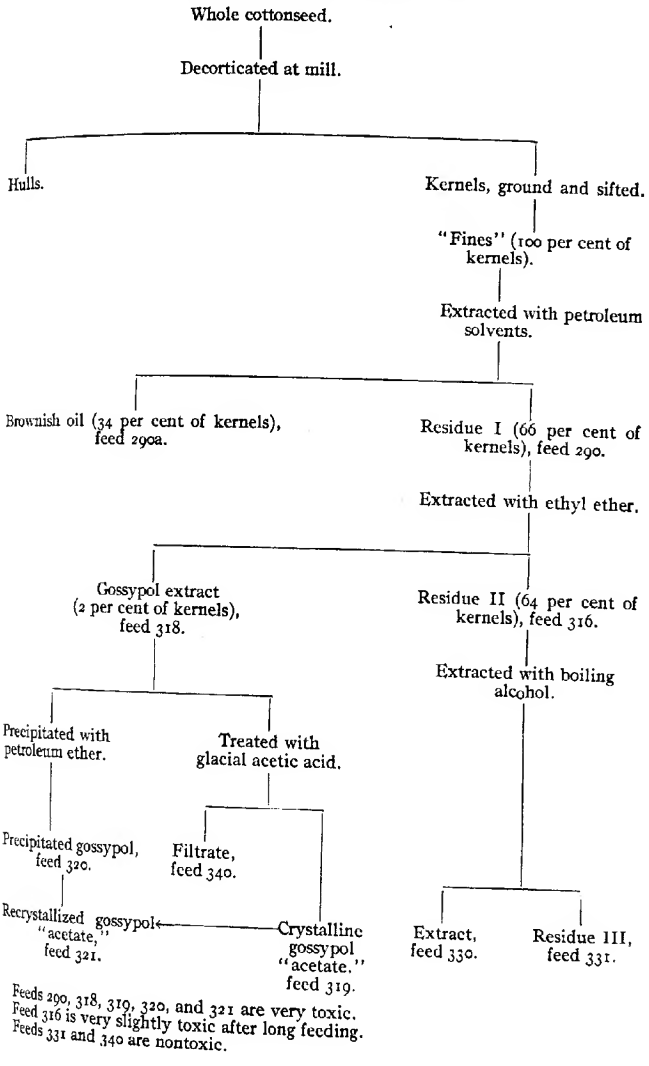
In our previous experiments we used cottonseed meal as the material for study, but in the experiments discussed in this paper we used cottonseed kernels as the initial substance, as gossypol is more readily and more completely extracted from the kernels than from the meal. Generally speaking, the meal and the kernels are toxic to rabbits to the same degree.

We extracted gossypol from ground cottonseed kernels with ethyl ether, after previously removing most of the oil with petroleum ether or gasoline. Gossypol was separated from the ethereal solution by evaporation, by precipitation with petroleum ether, or by precipitation with acetic acid.

These products, differing in purity, have been designated by us as "gossypol extract," "precipitated gossypol," and "gossypol 'acetate.'" All proved toxic to rabbits.

The method of preparing the gossypol and other feeds is shown in the accompanying outline.

OUTLINE OF METHOD OF PREPARING FEEDS



Feeds 290, 318, 319, 320, and 321 are very toxic.
Feed 316 is very slightly toxic after long feeding.
Feeds 331 and 340 are nontoxic.

OCCURRENCE AND PROPERTIES OF GOSSYPOL

If the cottonseed kernel is examined with a lens, many small yellowish brown to black spots may be seen (Pl. XXV). They are referred to by Hanausek (1907, p. 367) as "secretion cavities" in the following statement:

> Distributed among the mesophyll cells [of the cotyledons] are procambium bundles and globular, lysigenous **secretion cavities** (*se*) 100—400μ diameter. The lysigenous character of these cavities when mature is quite clearly evident. The tissue which surrounds them consists, in its outer portion, of tangentially flattened, very thin-walled cells, and within the last a mucilaginized layer in which the traces of the cell walls are still evident. This colorless mucilage layer, which treatment with hydrochloric acid and, after washing with water, with potash brings out as a yellow, folded, and laminated mass, encloses the greenish-black, opaque secretion (*v*). Since the mucilage layer is soluble in water, the secretion flows out from the sections laid in water in the form of a thick emulsion consisting of a colorless mass containing minute dark-colored grains (resin?) in lively molecular motion. Chlorzinc iodine colors the secretion red-brown, sulphuric acid dissolves it to a thick turbid fluid of a blood-red color. Ammonia colors the liquid greenish yellow without destroying the emulsion. Potash also imparts a green color.

They are designated by Watt (1907, p. 56) as "gland dots" and by Balls (1912, p. 13) as "resin glands." From these glands we have extracted gossypol and for clearness have alluded to them as gossypol glands. Their function does not seem to be very well known.

They occur in all parts of the cotton plant and in all varieties which we have seen. They are very abundant in the cambium layer of the bark of the cotton root.[1]

Gossypol was first isolated by Marchlewski (1899) from the "foots" in the purification of cottonseed oil, and on account of its source and phenolic properties he proposed for it the name "gossypol," from Gossyp[ium phen]ol.

Previous to Marchlewski's work the crude substance constituting the coloring matter of cottonseed oil was referred to by the older writers—e. g., Hanausek (1903, p. 755)—as "gossypin,"[2] which is described as a light-brown pungent powder.

Marchlewski (1899) proposed for gossypol the formula $C_{13}H_{14}O_4$, with $C_{32}H_{34}O_{10}$ as an alternate formula. Among its properties as described by him are the following: A beautifully crystalline yellow-colored dihydroxy phenolic substance, easily soluble in alcohol, benzene, chloroform, ether, acetone, and glacial acetic acid; insoluble in water; soluble in concentrated sulphuric acid with a magnificent red color; easily soluble in alkalies, the solution for the first second being yellow, after a short time becoming a beautiful violet and then fading, the changes being due to oxidation. The alcoholic solution gives a dark-green color with ferric

[1] Thus, we have an indication that gossypol may be the active principle of the medicinal extract of cotton-root bark. (Bouchelle, 1840.)

[2] The original work on gossypin has not been located by us.

chlorid. The samples dried at 125° to 130°, melted at 179° to 180°, and air-dried samples melted with quick heating at 188°.

Our experiments indicate that the substance which Marchlewski named "gossypol" contained acetic acid in combination with the substance to which we think the name "gossypol" should be assigned. The acetic-acid content of our different products varied from 8.5 to 9.5 per cent, depending upon the conditions under which crystallization took place. The substance containing acetic acid and the substance freed of acetic acid differ in elementary composition and in melting point, as one would expect. Marchlewski's empirical formulæ for gossypol appear to us to be erroneous, as they were based upon the ultimate analysis of the acetate instead of the substance freed from acetic acid.

Marchlewski supposed that gossypol might prove of value as a dyestuff, and before the publication of his article took out patents [1] to protect his discoveries. He made no suggestion as to its physiological activity, nor have we been able to find that anyone else has done so.

EXPERIMENTAL WORK WITH GOSSYPOL

METHOD OF ROUTINE FEEDING

Rabbits and guinea pigs were used in our experiments. Rabbits do not eat cottonseed meal nor cottonseed kernels readily. Therefore, to make the various solid feeds palatable, we moistened them with the best grade of molasses, rabbits eating the various feeds with great relish until made sick. They were fed liberally with green feed once a day.

In case of forced feeding a catheter was inserted to the stomach and the dose allowed to drain in. The intraperitoneal injections were made by the Station veterinarian, Dr. G. A. Roberts, by whom also the post-mortem examinations and notes were made.

The rabbits were fed in galvanized-iron cages, about 20 inches long by 16 inches wide by 10 inches deep. Each contained a trough with separate compartments for water and feed.

TOXICITY OF COTTONSEED KERNELS (FEED 290)

Cottonseed kernels were extracted with petroleum ether, which does not remove gossypol in appreciable quantities. A rabbit was started on 15 gm. daily of this feed, but it would not eat all of it. Diarrhea resulted on the second day, and its appetite for green feed was affected on the third and fourth days. It gradually ate less and less, so that the feed was discontinued on the eleventh day and the ether-extracted kernels (feed 316) substituted on the day following. During the last five days it ate only 11.5 gm. It ate 56.5 gm. of feed 290, losing 130 gm. in weight, but recovered on feed 316.

[1] English patent No. 24418 of 1895 and German patents Nos. 98074 and 98587 of 1898.

Two guinea pigs, A and B, were tried with this feed. Guinea pig A was off its feed at the time from eating precipitated gossypol spread on corn meal (feed 318). An attempt was made to give it kernels in which the gossypol was not so easily detected, but the animal would not touch them.

Guinea pig B had eaten feed 316 for 50 days and had gained in weight. After it had been on corn meal and molasses (feed 317) for about a week, it was placed upon feed 290 (7 gm. of kernels with molasses). It ate only 4 gm. of the kernels, although other feed was withheld for a day. We concluded from this that even the 4 gm. had affected it physiologically and had made it suspicious of the feed. After a week upon control feed, it ate feed 316 without objection.

Rabbit 957, which had eaten feed 316 for 46 days without noticeable effect, was rested for three weeks and then fed the residue after petroleum-ether extraction, which does not remove the gossypol. Its appetite was perceptibly affected on the third day, but it ate most of the feed for 6 days. On the ninth day it refused to eat feed 290, but ate green feed slowly. It died on the fourteenth day, showing symptoms of cottonseed-meal poisoning. See Table I.

TABLE I.—*Results of feeding cottonseed kernels (fat-free; feed 290) and cottonseed meal to rabbits and guinea pigs*

Feed and animal No.	Weight of animal.			Weight of feed eaten.		Number of days fed.	Result.
	Initial.	Final.	Loss.	Actual.	As kernels.		
Cottonseed kernels:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
Rabbit 958.....	1,560	1,430	130	56.5	85	11	Made sick and refused to eat.
Guinea pig A.....	680	.....	.....	0	0	1	Refused feed entirely.
Guinea pig B.....	650	.....	.....	4	6	1	Refused the feed.
Rabbit 957.....	1,800	1,535	235	100	150	14	Died.
Cottonseed meal:[a]							
Average for 22 rabbits.	1,577	1,238	339	[b] 133	.....	13	All died.

[a] The results of Withers and Brewster's experiments (1913) with cottonseed meal are here inserted for comparison.

[b] Each rabbit consumed from 48 to 225 gm. of cottonseed meal and died upon the feed in from 6 to 22 days.

TOXICITY OF GOSSYPOL EXTRACT

It is much simpler to prepare gossypol from cottonseed than from the oil.[1] Qualitative tests of ground cottonseed showed that gossypol could be extracted with ether, carbon bisulphid, chloroform, benzene, alcohol, but not with petroleum ether or gasoline. By extracting the

[1] This point will be discussed under the chemistry of gossypol, which will appear in a subsequent publication.

ground kernels in a Soxhlet apparatus for several hours with petroleum ether and then with ethyl ether we obtained a product which for convenience we called "gossypol extract." After the evaporation of the ether there was left a red resinous material which had a peculiar pungent odor and which amounted to about 2.5 per cent of the weight of the kernels used. This material seems to consist largely of gossypol, although we have not yet made an examination with reference to identifying other constituents. No doubt considerable oil is present.

Gossypol extract administered intraperitoneally and fed in one large dose in oil or in small daily doses with corn meal and molasses was found to be toxic to all the animals experimented with.

Catheter Feeding of Gossypol Extract

This gossypol extract from 90 to 180 gm. of cottonseed kernels was fed to each of four rabbits and proved fatal in every case. Care was taken to remove all the solvent, and the gossypol extract was dissolved in cottonseed oil which had been purified in this laboratory. The oil solution was then fed through a catheter. The control animal, on a large dose of cottonseed oil, had diarrhea the next day, but was normal thereafter. Table II summarizes the results obtained with the gossypol extract fed forcibly to rabbits.

Table II.—*Results of feeding gossypol extract and purified cottonseed oil with a catheter to rabbits*

Feed and rabbit No.	Weight of rabbit.	Weight of kernels before extraction.	Dose.	Result.
Gossypol extract:	*Gm.*	*Gm.*	*C. c.*	
923	1,500	90	15	Died in about 12 hours.
924	1,750	180	30 (½ water.)	Died in 30 to 40 hours.
926	3,000	About 160	30–35	Died in 25 hours.
927	2,100	170	30 (⅔ water.)	Died in less than 16 hours.
Purified cottonseed oil:				
925 (control)	2,500	.....	30–35	Sick with diarrhea next day only.

Post-mortem observations

Rabbit 923.—Part of dose still in stomach. First foot of intestines considerably injected. Excess serous fluid in abdomen, 10 c. c. No evidence of catheter reaching lungs.

Rabbit 924.—Lungs very much congested. Excess fluid in chest cavity, 3 to 4 c. c. Some hemorrhagic condition along blood vessels of large intestines.

Rabbit 926.—Lungs normal. Anus discolored from diarrhea.

Rabbit 927.—Lungs markedly congested.

INTRAPERITONEAL INJECTION OF GOSSYPOL EXTRACT

Cottonseed oil was used as the vehicle for the injection of the gossypol extract. This was readily available and of suitable consistency for injection. It was purified in this laboratory from a sample of crude oil. This oil was selected chiefly for its ability to hold the gossypol extract in solution. Crawford (1910, p. 531–532), under "Experiments with cottonseed oil," makes the following observations:.

After feeding a large dose of the crude cottonseed oil (25 c. c.) to a rabbit its weight steadily fell and remained low, and when a moderate dose (15 c. c.) was fed and this was followed by repeated small ones the animal died, showing irritation of the gastro-intestinal canal. Lendrich [1908] noted that after the daily administration of cottonseed oil his rabbits emaciated, but readily assimilated the same dose of oil that was given intraperitoneally.

After feedings with purified cottonseed oil, or with olive oil, there was a loss in weight, but the animals did not die. After feeding pure cod-liver oil the animals died. The loss in weight was small in the case of feeding purified cottonseed oil. The fact that the cottonseed oil gave no red reaction to litmus paper would suggest that the loss in weight, noted after feeding the crude oil, was not due to the free oleic acid. This acid has recently been shown to play an important rôle in the production of certain forms of anemia. Oils interfere with gastric digestion in man, and this fact must be taken into consideration in experiments on such animals as rabbits.

Two controls receiving purified cottonseed oil were affected to only a slight extent. All five rabbits receiving intraperitoneally an oil solution of gossypol extract died, the extract being the equivalent of from 45 to 85 gm. of cottonseed kernels. See Table III.

TABLE III.—*Results of intraperitoneal injection in rabbits of gossypol extract dissolved in purified cottonseed oil*

Feed and rabbit No.	Weight of rabbit.	Weight of kernels before extraction.	Dose.	Result.
Gossypol extract:	*Gm.*	*Gm.*	*C. c.*	
931	770	About 50	8	Died.
932	600	About 45	15 (½ water.)	Do.
934	937	85	5–6	Do.
928	1,090	85	7	Do.
929	1,225	About 50	4	Do.
Purified cottonseed oil:				
930 (control)	864	.....	10	Only slightly indisposed.
933 (control)	864	.....	10	Do.

POST-MORTEM OBSERVATIONS

Rabbits 931 and 932.—Fatal with complications in four days. Entire belly (subcutaneous) very edematous. Part of dose was injected subcutaneously.

Rabbit 934.—Died between the seventh and the nineteenth hour. Considerable serous fluid in abdomen. Serous fluid in chest cavity, 2 to 3 c. c.

Rabbit 928.—Fatal in three hours. Excess discolored serous fluid in abdomen containing oily globules. Moderate injection in intestines at points. Slight excess of fluid. Lungs slightly congested and slightly edematous.

Rabbit 929.—Died during night between the second and the thirteenth hour. Excess brownish serum in abdominal cavity. Small intestines show areas of marked injection. Lungs congested and somewhat edematous.

Rabbit 930.—Slightly indisposed on following day and normal thereafter. Appetite only slightly affected.

Rabbit 933.—Same as 930.

Feeding Gossypol Extract with Corn Meal and Molasses

An artificial cottonseed meal was made by pouring the concentrated ether extract of cottonseed kernels over corn meal. The daily feed for each of four rabbits was estimated to be equivalent to 30 gm. of cottonseed kernels, and for each of two others, 15 gm. Control animals were given corn meal and molasses. All the animals were supplied liberally with green feed (pea vines, cabbage, and collards) in the morning. In the afternoon (4 or 5 p. m.) they were given the various feeds mixed with molasses. The controls on corn meal and molasses did well, gained in weight, and need not be further mentioned. The gossypol extract proved very toxic. The animals receiving the equivalent of 30 gm. of cottonseed kernels refused to eat the cottonseed feed after the fifth day. They began to refuse green feed later, became sicker, and the last one died within 15 days. The two rabbits and a guinea pig receiving smaller doses were soon made sick. One rabbit and a guinea pig refused the feed thereafter, and the other rabbit died. See Table IV.

Table IV.—*Results of feeding gossypol extract (feed 318) with corn meal and molasses to rabbits and guinea pig* [a]

Daily feed equivalent to 30 gm. of cottonseed kernels

Feed and animal No.	Weight of animal.			Weight of mixture eaten.		Number of days fed.	Result.
	Initial.	Final.	Loss.	Actual.	As kernels.		
Gossypol extract with corn meal and molasses:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
Rabbit 941...	1,535	1,255	280	52	104	8	Died.
Rabbit 942...	1,605	1,250	355	54	108	12	Do.
Rabbit 943...	1,530	1,180	350	70	140	11.5	Do.
Rabbit 944...	2,095	1,595	500	71	142	15	Do.
Average...	1,691	1,320	371	62	124	11.7	Do.

[a] 1 gm. of the mixture of feed 318 and dry corn meal is equivalent to approximately 2 gm. of cottonseed kernels.

TABLE IV.—*Result of feeding gossypol extract (feed 318) with corn meal and molasses to rabbits and guinea pig*—Continued

DAILY FEED EQUIVALENT TO 15 GM. OF COTTONSEED KERNELS

Feed and animal No.	Weight of animal.			Weight of mixture eaten.		Number of days fed.	Result.
	Initial.	Final.	Loss.	Actual.	As kernels.		
Gossypol extract with corn meal and molasses:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
Rabbit 953...	1,915	1,755	160	41	82	11	Died.
Rabbit 954...	1,790	1,740	50	80	160	20	Experiment discontinued.
Gossypol extract alone:							
Guinea pig A	770	650	120	34	68	29	Do.

POST-MORTEM OBSERVATIONS

Rabbit 941.—Reddish serum in abdominal cavity, 15 c. c. Cecum deeply injected. Liver congested. Lungs slightly congested and edematous. Conspicuous thrombus in right auricle.

Rabbit 942.—Excess abdominal fluid, 15 c. c. Hemorrhagic (inflamed) and ulcerated condition at pyloric end of small intestines. Large thrombus in right auricle.

Rabbit 943.—Slight excess of abdominal fluid. Large intestines had some hemorrhagic areas. Liver congested.

Rabbit 944.—Reddish serum in abdomen, 25 c. c. Serous membrane injected. Small intestines reddened. Small thrombi present. Death due to enteritis.

Rabbit 953.—Mesenteric blood vessels injected. Viscera practically normal. Liver much congested. Kidneys much congested.

Rabbit 954.—Experiment discontinued because animal refused to eat feed 318 after the eighth day. Subsequently put on precipitated gossypol.

Guinea pig A.—Experiment discontinued because animal refused to eat feed 318.

In order to ascertain the effect of a large dose, a large healthy rabbit (945) was taken from the control feed and given all of feed 318 that it would eat. It consumed 40 gm., equivalent to 80 gm. of kernels, on the first day and was made sick on the following day. When it began to recover on the fourth day it was given a small feed and died on the ninth day, having lost considerably in weight. The protocol of rabbit 945 is as follows:

September 23, p. m.—Ate 40 gm. of feed 318 with molasses.

September 24.—Appears sick; has diarrhea. Ate little green; refuses feed 318.

September 25.—Seems indisposed; refuses feed 318.

September 26.—Better; eats cabbage. Weight 2,700 gm. Given 15 gm. of feed 318 and 15 gm. of corn meal with molasses. Ate equivalent to 7 gm. of feed 318.

September 27, 28, and 29.—Eats pea vines readily.

September 30.—Refuses green; p. m., ate corn meal and molasses readily.

October 1, a. m.—Refuses green. Died ninth day about 3 p. m. Weight, 2,410 gm. Post-mortem examination showed considerable excess fluid in abdominal cavity.

TOXICITY OF PRECIPITATED GOSSYPOL

By the term "precipitated gossypol" we designate a product obtained from the gossypol extract. In securing the extract in larger quantities the oil was not entirely removed from the cottonseed kernels by several previous extractions with gasoline; hence, the gossypol extract contained considerable amounts of oil. The dark-red oily gossypol extract, after evaporation of the ethyl ether, was mixed with a large quantity of petroleum ether. Under some conditions a part of the gossypol precipitated in brown flocks, which could be separated easily by filtration. Under conditions of rapid precipitation these flocks would agglomerate and form a red resinous material. Both the light-brown powder and the red resinous material dissolved in ether very readily, giving a deep cherry-red solution.

Another artificial cottonseed meal was prepared by dissolving weighed quantities of precipitated gossypol in ether, pouring the solution over corn meal, and warming over a steam bath to drive off the ether. One gm. of precipitated gossypol was usually mixed with 50 gm. of corn meal. This proportion was based on the assumption that gossypol existed in cottonseed kernels to the extent of 2 per cent.

Our earlier estimate of 2 per cent appears to be too high. The largest yields of crystalline gossypol acetate secured from the extract were from 0.8 per cent to 1 per cent of the weight of the kernels. This probably represents nearly the entire amount present, as very little gossypol is dissolved by gasoline and little is left after ether extraction, judging by the slight toxicity of the residue.

Pouring the deep cherry-red solution over corn meal gave it a red color. When this was warmed over the steam bath, the color of the corn meal changed to a typical cottonseed-meal yellowish brown. No explanation is offered for this change; but it is evidently not due to oxidation, as the change begins at the bottom of the mixture, not at the surface.

This artificial meal was fed to six rabbits and proved fatal in every case We had difficulty in getting them to eat it after having been once made sick.

Rabbit 954 was taken from feed 318 (gossypol extract) and offered corn meal and molasses containing 0.37 gm. of precipitated gossypol. It ate an equivalent of 0.3 gm. of the precipitated gossypol by the second day and seemed slightly indisposed. A week later it was again put on this feed, at the rate of 0.2 gm. daily. The quantity of gossypol eaten in the first six days was, per day, 0.2, 0.2, 0.17, 0.10, 0, and 0.05 gm. It ate none after this, but became sicker and died six days later.

Rabbit 961 ate 0.9 gm. of precipitated gossypol mixed with corn meal and molasses. It was apparently normal the next day, but refused cabbage on the third day. Thereafter it ate green feed well, but seemed to have no appetite for corn meal and molasses except when very hungry.

A week after recovery it was started on feed 319 (precipitated gossypol on corn meal). We planned to have it eat 0.3 gm. of gossypol daily. The first week 0.38 gm. of precipitated gossypol was eaten, the second week 0.67 gm., and only 0.60 gm. thereafter, a total of 1.65 gm. Death ensued after 19 days. The animal ate feed 319 sparingly and very irregularly.

A young rabbit (962) was fed similarly at the rate of 0.14 gm. a day. By weeks it ate, respectively, 0.97, 0.15, 0.15, and 0.34 gm. of precipitated gossypol. It was normal after the first week and died on the twenty-ninth day.

A guinea pig refused to do anything more than nibble feed 318 (gossypol extract), eating in 29 days only 34 gm. of the feed. It could not be induced to eat feed 319 (precipitated gossypol) any better, consuming only 1.13 gm. in 27 days. The autopsy showed that a mesenteric twist had cut off the blood supply of the last half or third of the intestines, so that death was not directly traceable to the feed.

Rabbit 949 was fed a large dose (1.44 gm.) of the precipitated gossypol mixed with corn meal and molasses. The next two days it suffered from diarrhea and refused to eat this feed, but it ate green feed. Thereafter it was given precipitated gossypol in small doses, but it usually refused all or part of this. Steadily losing weight, the animal died after 35 days, having eaten a total of 4.47 gm. of gossypol, inclusive of the large dose. The amounts eaten each week were, respectively, 2.08, 0.58, 0.50, and 0.68 gm.

Rabbit 937 had previously eaten the ether-extracted residue (feed 316) for 61 days and had increased in weight. Then, after several days on corn meal and molasses the rabbit was fed precipitated gossypol. We planned to feed 0.3 gm. a day, but only on three days did it eat this amount, usually refusing it entirely or in part. After 21 days a crystalline product was substituted for precipitated gossypol. The animal steadily decreased in weight and died after 33 days. The total amount of gossypol consumed was 2.52 gm. By weeks, 1.19, 0.27, 0.5, 0.57, and 0 gm. of gossypol were consumed. It ate practically nothing during the last 8 days. See Table V.

TABLE V.—*Results of feeding precipitated gossypol with corn meal and molasses (feed 319) to rabbits and guinea pigs*

Animal No.	Weight of animal.			Weight of precipitated gossypol eaten.	Number of days fed.	Result.
	Initial.	Final.	Loss.			
	Gm.	*Gm.*	*Gm.*			
Rabbit 954	1,740	1,275	465	1.02	13	Died.
Rabbit 961	1,830	1,435	395	1.22	19	Do.
Rabbit 962	630	465	165	1.62	29	Do.
Guinea pig A	660	565	95	1.13	27	Do.
Rabbit 949	2,375	1,702	673	[a]4.47	35	Do.
Rabbit 937	2,890	1,925	965	2.50	33	

[a] This quantity (4.47 gm.) includes a large dose of 1.44 gm. which evidently passed the bowel quickly.

POST-MORTEM OBSERVATIONS

Rabbit 954.—Excess fluid in abdominal cavity. Serous membrane in icteric condition.

Rabbit 961.—Large excess abdominal fluid. Small intestines show enteritis. Blood vessels congested.

Rabbit 962.—Large excess abdominal fluid. Small intestines inflamed and hemorrhagic. Small thrombus in right heart.

Guinea pig A.—Evidently died from mesenteric twist (convolvulus) in intestines. Posterior third greatly inflamed. Lungs congested and edematous.

Rabbit 949.—Slight excess of abdominal fluid. Small intestines conspicuously inflamed. Large pericardial abscess present. Enteritis.

Rabbit 937.—Slight excess abdominal fluid. Small intestines irritated throughout. Conspicuous thrombi in heart. Lungs congested and edematous.

TOXICITY OF CRYSTALLINE GOSSYPOL "ACETATE"

Crystalline gossypol "acetate" was obtained from a gossypol extract by the action of glacial acetic acid, which caused a slow deposition of yellow crystals. We have designated this substance as an "acetate," although the acetic acid present is not firmly bound.[1] The product corresponded in general properties to Marchlewski's gossypol. It was administered intraperitoneally to four rabbits, proving fatal, and was fed daily to eight rabbits. It made all of them sick. One died from secondary causes. Two refused to eat the feed after 5 and 15 days, respectively, and five died within from 13 to 55 days, having eaten from 0.35 to 2.53 gm. of crystalline gossypol "acetate."

INTRAPERITONEAL INJECTION OF CRYSTALLINE GOSSYPOL "ACETATE"

We dissolved 1.2 gm. of gossypol "acetate" in ether and mixed the solution with 16 c. c. of cottonseed oil. The ether was evaporated by heating over a steam bath. This was given intraperitoneally to two rabbits of about 1,100 gm. weight so that each rabbit received from 0.5 to 0.55 gm. of gossypol "acetate." Both animals died and were cold in six hours. The autopsy showed a considerable portion of the dose in the abdominal cavity, so that much more than a lethal dose was given.

About 3 gm. of a yellow, crudely crystalline product similar to that which was injected in 0.5 gm. doses to rabbits 955 and 956 was recrystallized as follows: The material was dissolved in hot alcohol and heated to boiling, then 50 per cent of acetic acid was added until the liquid became slightly turbid. This mixture was again heated to the boiling point and allowed to cool. Most of the substance separated in yellow, flat, pointed crystals, about 0.1 to 0.5 mm. long, which melted with darkening at about 178° C.

[1] The term "acetate" is arbitrarily used. Gossypol crystallizes from glacial acetic acid and even from quite dilute acetic acid with a molecule of acetic acid, which is not removed by long boiling with water or by heating to 115° to 120°. Its presence thus escaped our attention as it did Marchlewski's. It is entirely improbable that a small amount of acetic acid modifies in any way the physiological action of gossypol. See "Results of feeding precipitated gossypol."

To prepare the injection, 0.7 gm. of this substance was dissolved in ether and the ethereal solution mixed with 20 c. c. of purified cottonseed oil. The clear reddish yellow solution was warmed over steam until it had not the slightest odor of ether. This was then injected in doses of 10 c. c. into two rabbits, 963 and 964, weighing 1,560 and 1,485 gm., respectively. In a few minutes the rabbits became very uneasy and then passed into a sort of stupor. Rabbit 963 died in 3.5 hours and 964 in 4.5 hours. The death of rabbit 964 was witnessed. Shortly before death it toppled over on its side, had several convulsions, gasped several times, squealed, and died.

In these cases, as in the previous one, there was considerable injecta left in the abdominal cavity. See Table VI.

TABLE VI.—*Result of administering crystalline gossypol "acetate" intraperitoneally in cottonseed oil to rabbits*

Rabbit No.	Initial weight of rabbit.	Weight of gossypol.	Dose volume.	Weight of gossypol per kilo of body weight.	Result.
		CRYSTALLINE GOSSYPOL "ACETATE"			
	Gm.	*Gm.*	*C. c.*	*Gm.*	
955	1,115	0.55	8	0.493	Died.
956	1,180	.55	8	.466	Do.
		RECRYSTALLIZED GOSSYPOL "ACETATE"			
963	1,560	0.35	10	0.244	Died.
964	1,485	.35	10	.235	Do.

POST-MORTEM OBSERVATIONS

Rabbits 955 and 956.—Dead and cold after six hours. Apparent nonabsorption of much of the injection. Excess of fluid. Peritoneum stained brown. Visceral blood vessels slightly injected.

Rabbit 963.—Died in convulsions. Part of injecta present as oily globules. Serum present also. Serous membrane stained yellow.

Rabbit 964.—Same as 963, except small intestines were rather markedly injected.

FEEDING CRYSTALLINE GOSSYPOL "ACETATE" TO RABBITS

Crystalline gossypol "acetate" with corn meal and molasses (feed 319) was fed to rabbit 965. The feed was refused on the fourth day, after which it was not further given. Only on the first day did the animal eat the entire amount fed. After eating 0.3 gm. of crystallized gossypol "acetate," it had a bad diarrhea and little appetite for green feed the next day. The protocol was as follows:

December 15, first day.—Ate 0.3 gm. with corn meal and molasses; weight, 2,340 gm.

December 16, second day.—Bad diarrhea, and eats little green feed.

December 16, p. m.—Ate 0.2 gm. of gossypol.
December 17, a. m.—Ate green feed well.
December 17, p. m.—Ate 0.17 gm. of gossypol.
December 18, a. m.—Ate green feed well.
December 18, p. m.—Refused to eat the "doped" food.
December 19, a. m.—Slightly sick; eats green feed moderately.
December 19, p. m.—Refused to eat corn meal and molasses, but ate green feed. Amount eaten, 0.67 gm.; final weight, 2,140 gm.; loss, 200 gm.
December 20 to December 31.—Ate green and corn meal and molasses; regained normal health.

Rabbit 951, weight 1,800 gm., which had previously stood two long feeding periods on ether-extracted cottonseed kernels, was fed crystalline gossypol "acetate." It ate 0.6 gm. in the first four days and then became sick, refusing all feed. On the tenth day it weighed 1,605 gm. From then till the twenty-eighth day, on which it died, it ate 0.32 gm. Weight about 1,170 gm.

Post-mortem observations: Teaspoonful excess in abdomen. Moderate injection of serous membranes. Some hemorrhagic areas in stomach. Mesenteric blood vessels more or less injected. Small thrombus in heart.

Rabbit 965A ate the same preparation of gossypol mixed with corn meal and molasses. It ate, by weeks, 0.64, 0.08, 0.5, 0.37, 0.07, 0.80, 0.31, and 0 gm.; total, 2.53 gm. This was a large healthy rabbit at the beginning. The post-mortem examination showed a slight excess of fluid in the abdominal cavity and serous membranes highly congested.

A new lot of rabbits was secured from a supply house in Washington, D. C. These rabbits were not as healthy and resistant as could be desired, some evidently having been used before in experimental work.

Rabbits 974, 976, 977, and 972 were given the same gossypol feed. Rabbit 974 ate 0.33 gm. of gossypol with corn meal and molasses. The next day it had a very bad diarrhea, which continued all day. It ate no green feed and only a little gossypol feed for the next four days, after which gossypol was withdrawn from the feed. On the nineteenth day it had not entirely recovered from the effects of eating 0.47 gm. of gossypol during the first five days. Loss in weight during 15 days, 330 gm.

Rabbit 976 was fed 0.25 gm. of gossypol. It had diarrhea the next day and no appetite. The third day it ate 0.05 gm.; fourth day, 0.09 gm.; fifth day, 0.01 gm.; and afterwards refused the gossypol feed. It lost in weight steadily until death, on the fifteenth day. Gossypol eaten, 0.40 gm. Loss in weight, 475 gm.

Rabbit 977 was fed like 976, with approximately the same effect. It died on the thirteenth day. Gossypol eaten, 0.35 gm. Loss in weight, 580 gm.

Rabbit 972 ate 0.55 gm. of gossypol and died in 13 days.

These last three rabbits were fed on a product which was somewhat darker in color than the gossypol given rabbit 974. The gossypol tends to take on a greenish or brown tinge under some conditions of prepara-

tion. Gossypol from old seeds is greenish. The post-mortem examination showed considerable irritation in the small intestines of these rabbits.

EFFECT OF SMALL DOSES OF CRYSTALLINE GOSSYPOL "ACETATE"

Rabbit 978, weight 2,100 gm., was fed on recrystallized gossypol "acetate" at the rate of 0.05 gm. daily mixed with corn meal and molasses. After one week it began to show a diminished appetite for the feed. On the nineteenth day it was given a double dose by mistake, and for two weeks thereafter showed a very poor appetite for the feed. At that time it weighed 1,820 gm.

Its record by weeks is as follows:

Quantity of gossypol eaten.....gm.. 0.34; 0.33; 0.335; 0.25; 0.25; 0.32; 0.125.

Weight of rabbit.....gm.. 2,045; 2,010; 2,070; 1,820; 1,930; 1,730.

Total quantity of gossypol eaten.....gm.. 1.95.

The animal died after 51 days. The post-mortem examination showed that a convolvulus had set up a necrotic condition in the intestine. Whether the feed was contributory to this condition we are unable to say. See Table VII.

TABLE VII.—*Results of feeding crystalline gossypol "acetate" (feed 319) to rabbits*

Feed and rabbit No.	Weight of rabbit.			Weight of gossypol "acetate" eaten.	Number of days fed.	Result.
	Initial.	Final.	Loss.			
	Gm.	*Gm.*	*Gm.*	*Gm.*		
Gossypol "acetate":						
965	2,340	2,140	200	0.67	5	Made sick.
951	1,800	1,170	630	.92	28	Died.
965A	2,265	1,600	665	2.53	55	Do.
Recrystallized gossypol "acetate":						
974	1,670	1,340	330	.47	15	Made sick.
976	1,670	1,195	475	.40	15	Died.
977	1,825	1,245	580	.35	13	Do.
972	1,425	1,115	310	.55	13	Do.
Gossypol "acetate" fed in small doses (0.05 gm. per day):						
978	2,100	1,730	380	1.95	51	Died from secondary causes.

FEEDING GOSSYPOL "ACETATE" TO FOWLS [1]

Two cockerels (986 and 987) previously fed on cottonseed meal to study the symptoms, were started on gossypol. Powdered gossypol in 0.3 gm. doses was fed, followed by a little water. On the fourth

[1] This experiment was carried on under the supervision of Dr. B. F. Kaupp, Pathologist, of the Poultry Division, North Carolina Agricultural Experiment Station.

day cockerel 986 had fallen off in weight, and his appetite was only fairly good. On the sixth day his digestion was poor, his crop being full of food. The bird steadily lost in weight until death on the sixteenth day, dropping from 3 to 2 pounds in weight. The bird was given 4.1 gm. of gossypol, at least 0.5 gm. of which was found in the crop after death.

The post-mortem examination showed extreme emaciation. Food in crop for a number of days; indications that gossypol interferes with the nervous mechanism of digestion. Diarrhea, the contents being fluid in rectum only. Semisolid in other portions. An absence of visible lesions.

Cockerel 987, slightly larger than 986, reacted in quite the same manner as 986 to administrations of gossypol. He steadily wasted away, falling from 3 pounds 8 ounces to 2 pounds 3 ounces, and died on the twenty-sixth day. Amount of gossypol fed, 5 gm.

The post-mortem examination showed extreme emaciation. Testes, spleen, gizzard, and other organs to a certain extent in a state of absorption.

Of chief interest to us was a statement by Dr. Kaupp to the effect that the gossypol produced the same results as cottonseed meal.

A healthy pullet (989) was started on gossypol. On the fourth day her digestion was affected. Nine doses of 0.3 gm. each in a period of 20 days were sufficient to cause her to refuse all feed and to waste away. She died on the thirty-sixth day, weight 1.5 pounds, just half the initial weight. Dr. Kaupp reported that "the autopsy revealed nothing beyond extreme emaciation." See Table VIII.

TABLE VIII.—*Results of feeding gossypol "acetate" to fowls*

Fowl No.	Weight of fowl.			Weight of gossypol "acetate" eaten.	Death occurred in—
	Initial.	Final.	Loss.		
	Pounds.	*Pounds.*	*Pounds.*	*Gm.*	*Days.*
986	3.0	2.0	1.0	4.6	16
987	3.5	2.2	1.3	5.0	26
989	3.0	1.5	1.5	2.7	36

FEEDING GOSSYPOL "ACETATE" TO A PIG

Pig 989, weighing 21 pounds, was fed corn meal and molasses. He ate with relish. About 5 p. m. he was given 3 gm. of crystalline gossypol "acetate" mixed with 80 gm. of corn meal and molasses, the whole feed weighing about 125 gm. He ate all but a small part. The next morning he had little appetite. In the afternoon he was given 1 gm. of gossypol on corn meal and molasses, most of which was left on the following morning. The remainder was made into slop. He ate part of this. On the afternoon of the same day he vomited; the following morning he appeared sick. We were unable to continue this experiment.

TOXICITY OF GOSSYPOL EXTRACT FREED OF GOSSYPOL (FEED 340)

Gossypol extract was treated with acetic acid for the preparation of gossypol "acetate," as previously described. The precipitate contained most of the gossypol. The filtrate, which contained only a small amount of it, was mixed with corn meal and dried. The extract, thus practically freed of gossypol, was fed to two rabbits in very large amounts and produced no symptoms of poisoning in either.

The rabbits weighed 1,995 and 1,986 gm., respectively. Each was fed the extract from 500 gm. of kernels during five days, the daily amounts for the first two days corresponding to 130 gm. each and for the three other days, 90 gm. each. No rabbit could have eaten within this short period without fatal results such a large amount of kernels or the gossypol from them.

TOXICITY OF OXIDIZED GOSSYPOL (FEED 338)

Withers and Ray (1913b) noted that the toxicity of cottonseed meal could be destroyed by boiling with alcoholic caustic soda. The alkaline alcoholic filtrate from this treatment was also found to be nontoxic, owing to the oxidation of the phenolic gossypol to an organic acid. To ascertain the correctness of this view, weighed amounts of recrystallized gossypol dissolved in alcohol were treated with dilute caustic soda. The solution was exposed to air overnight, made slightly acid with hydrochloric acid, and evaporated to dryness. The residue was mixed with corn meal and molasses for feeding. The substance had a pronounced bitter taste. Two small rabbits ate the oxidation product, equivalent to 3 gm. of gossypol apiece, in the course of 16 days without the slightest sign of being affected thereby. See Table IX.

TABLE IX.—*Result of feeding oxidized gossypol to rabbits* [a]

Rabbit No.	Weight of rabbit.			Equivalent in gossypol of feed eaten.
	Second day.	Fifteenth day.	Gain.	
	Gm.	*Gm.*	*Gm.*	*Gm.*
983	1,280	1,420	160	3
984	850	1,065	215	3

[a] On 4 days out of the 16 oxidized gossypol was not fed.

TOXICITY OF KERNELS WITH GOSSYPOL INCOMPLETELY EXTRACTED

ETHER-EXTRACTED KERNELS (FEED 316)

Decorticated cotton seeds were secured from Charlotte, N. C. They were sifted to remove as much lint and hulls as possible. The kernels were then ground in a mill and sifted through an 18- to 20-mesh sieve and

extracted for five to eight hours with ethyl ether in a filter-paper thimble in a large Soxhlet apparatus.[a] After extraction the residual ether was evaporated and the kernels sifted through a 1-mm. sieve. They were either heated for an hour or so over a steam bath or dried in the air.

Sixteen rabbits and two guinea pigs were fed upon ether-extracted kernels. One of the rabbits had its back broken on the fifteenth day and was chloroformed. It showed none of the usual symptoms of cottonseed-meal feeding. Nine of the animals (Table X, part 1) died in from 19 to 75 days and 8 (Table X, part 3) were alive and normal at the end of the feeding experiments, which ranged from 42 to 71 days. Calculated to the average daily equivalent of kernels per kilogram of initial live weight, 9.4 gm. of ether-extracted kernels proved lethal to nine rabbits after 45 days, while 11.5 gm. did not prove lethal to eight others after 52 days. The ether-extracted kernels are therefore much lower in toxicity than cotton seed meal, of which a daily feed of 6.5 gm. per kilogram for 13 days was found lethal by Withers and Brewster (1913).

In view of the strikingly positive results obtained with ether extract and gossypol isolated therefrom, it was naturally expected that the ether-extracted kernels would prove nontoxic. With death resulting to only 9 out of 17 animals, and then not until after an average of 45 days, it is not unlikely that if the ether-extracted kernels had been fed in as small quantities as the cottonseed meal (6.5 instead of 9.4 gm.) they would have proved practically nontoxic, as anticipated.

The thoroughness of extraction is very important, as shown by the fact that kernels through which ether had only percolated proved toxic in from 11 to 14 days (Table X, part 2), while the average lethal period for kernels extracted from five to eight hours (Table X, part 1) was 45 days, or more than three times as long (Table X).

TABLE X.—*Results of feeding ether-extracted cottonseed kernels (feed 316) to rabbits*

PART 1

Animal No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.		Number of days fed.	Result.
	Initial.	Final.	Gain or loss.		Total.	Daily.		
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
940	2,100	1,655	−455	348	522	14	35	Died.
935	1,380	1,500	+120	568	852	11	75	Do.
952	1,480	1,678	+198	251	373	20	19	Do.
947	1,875	1,576	−300	605	909	17	53	Do.
958	1,430	1,318	−112	494	741	15	52	Do.
959	2,560	2,165	−395	882	1,323	21	64	Do.
Guinea pig B (second period)[b]	610	535	− 85	199	300	9	34	Do.
970	1,515	1,670	+155	285	420	21	20	Do.
974	1,510	1,525	+ 15	458	687	14	50	Do.

[a] Before the ether extraction the ground kernels were extracted with petroleum ether or gasoline in case it was desired to work up the ether extract for gossypol.

[b] Rabbit 651 and guinea pig B were fed for two separate periods, there being a rest of two weeks between the two periods.

TABLE X.—*Results of feeding ether-extracted cotton seed kernels (feed 326) to rabbits*—Continued

PART 2

Animal No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.		Number of days fed.	Result.
	Initial.	Final.	Gain or loss.		Total.	Daily.		
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
939	1,510	1,255	−255	[a] 89	133	12	11	Died.
938	1,930	1,405	−525	[a] 70	105	8	13	Do.
936	1,415	955	−460	[a] 69	103	7	14	Do.

PART 3

937	2,630	2,900	+270	1,013	1,520	25	61	Lived.
951 (first period)[b]	1,800	1,990	+190	609	914	22	42	Do.
951 (second period)[b]	1,940	1,790	−150	539	810	15	53	Do.
957	1,650	1,835	+185	627	942	21	46	Do.
Guinea pig B (first period)[b]	620	685	+ 65	320	480	10	50	Do.
960	2,040	2,195	+155	198	300	20	15	Chloroformed.
969	1,475	1,797	+322	798	1,197	16	71	Lived.
981	1,890	2,230	+340	880	1,320	30	44	Do.
985	1,315	1,730	+415	615	923	18	51	Do.

[a] Feed percolated only with ether.

[b] Rabbit 951 and guinea pig B were fed for two separate periods, there being a rest of two weeks between the two periods.

Rabbits 939, 938, 936, and 940.—The post-mortem examination showed symptoms resembling cottonseed-meal poisoning.

Rabbit 935.—When this animal had recovered from the effects of the incompletely extracted kernels, it weighed 1,030 gm. It ate 647 gm. in 49 days and then weighed 1,600 gm. It died on the seventy-fifth day, showing symptoms other than those common to cottonseed-meal poisoning.

Rabbit 937.—Slightly off its feed in the middle of the experiment, but was in perfect condition when this feed was discontinued.

Rabbit 952.—Post-mortem examination showed a small amount of excess abdominal fluid and the small intestines considerably congested. Death due to enteritis. Symptoms of cottonseed-meal poisoning.

Rabbit 951 (first period).—Kept in good condition most of the time.

Rabbit 951 (second period).—Somewhat affected by feed. Ate but lightly at end.

Rabbit 947.—About 40 c. c. excess serous fluid in abdominal cavity. Considerable necrosis had set up.

Rabbit 957.—Perfectly well at the end of the experiment and remained so during the subsequent three weeks. It acquired no "immunity" toward cottonseed poisoning, however. See data on rabbit 957 on feed 290.

Rabbit 958.—Put on this feed after being made sick on unextracted kernels (feed 290). Post-mortem examination showed about 15 c. c. excess serous fluid in abdomen; small intestines markedly injected with slight hemorrhagic areas; liver congested; large abscess in submaxillary lymphatic glands.

Rabbit 959.—Began to be affected on the forty-seventh day, having gained up to this date. The post-mortem examination showed excess bloody serum in abdominal cavity; large amount of serum present with coagulated fibrin; serous membranes congested.

Guinea pig B.—In perfectly normal health at the end of first feeding period. Died in second experiment, showing much irritation in intestines.

Rabbit 960.—Broke its back accidentally and was chloroformed. Its case is of interest in that the autopsy showed no pathological lesions in the time usually required to kill an animal with cottonseed meal.

Rabbits 969 and 970 —Had previously been on the alcoholic extract (feed 330) for 26 days without ill effects.

ARE THE BAD EFFECTS OF FEED 316 DUE TO GOSSYPOL?

Feed 316 is of a pale-yellow color. Moistened with ether and examined through a lens, numerous black specks are seen, as in the unextracted kernels. These represent the gossypol glands, the contents of which have in part been removed by ether. Sometimes these glands have become separated from the seed tissue and can be examined individually. They dissolve in concentrated sulphuric acid with a red color, indicating gossypol. On warming a gram or so of the extracted kernels with alcoholic potash and shaking, a darkening in shade with a suggestion of a purple color takes place in the supernatant liquid. This is characteristic of gossypol, the depth of color depending upon the amount of gossypol. When the alcoholic alkali first touches the particles, they turn several shades deeper to a yellow that matches the color of cottonseed meal very closely. This is also characteristic of gossypol. On the addition of acid the former light-yellow color returns.

If the extracted kernels are allowed to soak in water for a short while, a substance dissolves which gives the liquid a reddish violet color. This is probably due to an oxidation product of gossypol. The coloration is quite permanent.[1]

These experiments show that gossypol or oxidation products of gossypol or possibly other similar substances (see Power and Browning, 1914, p. 420) are still present in this residue after the long-continued ether extraction.

The fact that gossypol is not completely extracted by ether, although very soluble in it, may be due to its being held mechanically in impervious cells, being fixed dye like in the tissue, or being in the form of an insoluble metallic salt.

Therefore, it seems to us that even the slight toxicity of the residue after ether extraction is due to its gossypol content. (See data on rabbit 978, Table VII, p. 276.)

TOXICITY OF KERNELS PRACTICALLY GOSSYPOL-FREE

ETHER-ALCOHOL-EXTRACTED KERNELS (FEED 331)

In order to determine whether it were possible by extraction with solvents to prepare a cottonseed feed which would not produce any bad results with rabbits, the ground kernels were extracted first with gasoline to remove oil, etc., then with ether in a large separatory funnel until the percolate was of a very faint-yellow color. The residue was

[1] An attempt will be made to correlate this observation with the red sap (anthocyan?) of certain species of Gossypium.

removed and boiled in a large flask with alcohol. The first alcoholic extracts were quite highly colored. The extraction was repeated until a filtrate was obtained which possessed only a pale-yellow color.

The ether-alcohol-extracted kernels were fed daily to three rabbits for from 72 to 105 days in amounts ranging from the equivalent of 15.2 to 24 gm. of kernels; at the end of the period the rabbits were normal and all had gained from 30 to 148 per cent of their initial weight and were still gaining.

The severe test that these rabbits endured is sufficient to show that a feed has been prepared which can be called practically nontoxic.

It also indicates that protein and organic phosphates (inosite phosphoric acid salts), which are present in the feed in larger amounts than in cottonseed meal, have very little, if anything, to do with cottonseed-meal poisoning.

TABLE XI.—*Results of feeding cottonseed kernels extracted with gasoline, ether, and alcohol (feed 331) to rabbits*

Rabbit No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.		Number of days fed.
	Initial.	Final.	Gain.		Total.	Daily.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
966	1,335	1,897	562	1,043	1,738	24	72
967	640	1,590	950	957	1,595	15.2	105
968	1,610	2,095	485	1,108	1,846	19.6	94

Rabbit 967 was slightly off its feed only on the fortieth and forty-first days, but recovered quickly and continued to gain. See Table XII.

Rabbit 968 was one of the lot of Belgian hares received from Washington, D. C., in rather poor health. It was started at the rate of 15 gm. daily, equivalent to 25 gm. of whole kernels. This proved too heavy feeding, for after two weeks the animal went off its feed for several days. The ration was then reduced (Table XII).

TABLE XII.—*Record of rabbits 967 and 968 on feed 331*

Rabbit No. and period (10-day).	Weight of feed eaten.	Weight of rabbit.	Rabbit No. and period (10-day).	Weight of feed eaten.	Weight of rabbit.
	Gm.	*Gm.*		*Gm.*	*Gm.*
Rabbit 967	.....	640	Rabbit 968	.....	1,610
1	62	705	1	140	1,525
2	70	845	2	85	[a] 1,410
3	73	930	3	81	[a] 1,460
4	93	.....	4	100	[a] 1,640
5	61	[a] 1,055	5	100	[a] 1,725
6	96	[a] 1,200	6	107	[a] 1,900
7	100	[a] 1,335	7	135	[a] 2,105
8	100	[a] 1,420	8	150	[a] 2,130
9	122	[a] 1,515	9	150	[a, b] 1,890
10	120	[a] 1,560	Last 4 days	60	2,095
Last 5 days	60	[a] 1,590			

[a] 10 gm. of corn meal was added daily to the feed.
[b] Loss in weight was due to the delivery of seven young rabbits.

TOXICITY OF AN ALCOHOLIC EXTRACT OF GASOLINE-ETHER-EXTRACTED KERNELS (FEED 330)

The solution obtained by treating gasoline-ether-extracted cottonseed kernels with hot alcohol was evaporated to a small volume over a water bath. The extract was about 10 to 12 per cent of the kernels. As the solution was concentrated, it separated into a yellowish layer (probably chiefly raffinose) and a reddish black resinous layer. The concentrated solution was mixed with corn meal, dried, and pulverized. This feed had a yellow-brown color and a very bitter taste. It was fed to two rabbits (969 and 970) in amounts equivalent to 50 gm. of cottonseed daily. It did not prove to be toxic, although the rabbits lost slightly in weight and frequently left part of their feed, possibly on account of its bitter taste. On the fourth day of feeding a slight diarrhea was noticed in both animals. They were quite normal after having been on the feed for 26 days, when it was discontinued (Table XIV).

TABLE XIV.—*Results of feeding an alcoholic extract of gasoline-ether-extracted cottonseed kernels (feed 330) to rabbits*

Rabbit No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels	Number of days fed.	Result.
	Initial.	Final.	Loss.				
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
969	1,530	1,475	55	243	1,000	26	Lived.
970	1,650	1,515	135	214	900	26	Do.

These two animals were then fed on the material from which the extract was obtained (see feed 316).

The presence of some gossypol due to the incomplete extraction by ether doubtless causes the slight toxicity of feed 316.

The nontoxicity of feed 330 may be explained on the assumption that the gossypol, extracted from feed 316 by alcohol, undergoes oxidation during the process of extraction or evaporation. This point needs further study (see feed 338).

Both the alcoholic extract and oxidized gossypol possess a bitter taste, whereas gossypol and gossypol "acetate" are tasteless and odorless.

ARE OTHER TOXIC SUBSTANCES PRESENT?

Although the feeding experiments show that gossypol is very poisonous, produces symptoms of cottonseed-meal poisoning, and affords a satisfactory explanation of the toxic properties of cottonseed meal, we do not claim to have made a complete study of the cottonseed from the standpoint of toxicity. The following problems are still unsolved:

(1) To exactly what extent does gossypol occur in cottonseed—i. e., in the petroleum extract and in the ether-extracted residue—and is gossypol the only toxic substance of like nature in the gossypol extract?

(2) To what extent, if any, do other toxic substances not related to gossypol contribute to the total action of cottonseed meal—i. e., are decomposition products and toxic alkaloids present in cottonseed meal? In this connection it may be stated that Friemann (1909) found an unidentified alkaloid in cottonseed meal, which caused paralysis of exposed frogs' hearts. Werenskiold (1897) obtained from cottonseed meal an alkaloid for which he proposed the name "gossypein." He also found betain and cholin. Withers and Fraps (1901, p. 81) state:

> Gossypein, if present in the sample tested, was present in very minute quantity. The filtrate from 363 grams cottonseed meal, ready for precipitation with phosphotungstic acid, was extracted with chloroform, and nitrogen was determined in the extract. It was equivalent to 0.008 per cent gossypein (calculated as cholin).

Withers and Ray (1913b) state:

> No evidence was found of the presence of toxic alkaloids in the feed, or of hydrocyanic acid in the feed or in the bodies of animals dead from eating cottonseed meal.

The fact that many solvents acting on *cottonseed meal* failed to remove the toxic substance suggests the possibility that in the manufacture of cottonseed meal the gossypol in the glands is fixed dyelike in the tissue of the seed, so that solvents like ether, in which gossypol is easily soluble, do not completely extract it. Gossypin is said to dye wool and silk (proteid materials). (See p. 265.) Again, some of the glands may be made impervious to the action of solvents by the mucilaginous substance surrounding the secretion. As is well known, cottonseed contains a large amount of raffinose (4 to 6 per cent). In the manufacture of the meal—e. g., in steaming—this may be partly dissolved and subsequently a film of this sugar deposited on the particles of meal. These factors must be considered with reference to the nonremoval of gossypol from the meal by solvents.

It may be noted that every gram of extracted residue represents at least 1.5 gm. of kernels. A ration of 15 gm. per day means that the animal eats all the protein and practically all the phosphorus of 22.5 gm. of seeds.

The residue (feed 316) is rich in nitrogen and ash. The values of nitrogen, protein, sulphur, and phosphorus in the ground kernels, and in feeds 316 and 331 are given in Table XV.

TABLE XV.—*Percentage of nitrogen, protein, sulphur, and phosphorus in ground cottonseed kernels and in feeds 316 and 331*

Feed.	Nitrogen.	Protein.	Sulphur.	Phosphorus.
Ground kernels	5.24	32.7	0.40	.....
Feed 316	8.6	53.7	.54	1.2
Feed 331	8.8	55.0	.....	.....

It is quite probable that the animal organism is able to take care of the large amount of proteins and phosphorus compounds, as may be inferred from the results of feed 331.

The latest published endeavor to ascribe the poisonous effects to a specific chemical substance was by Crawford (1910), whose experiments seemed to point to salts of pyrophosphoric acid.

The improbability of this conclusion was shown by Withers and Ray (1913a), of this Station, in feeding experiments. Cottonseed meal was extracted with ammonium citrate. This left an insignificant amount of phosphorus in the residue, which was almost as toxic as whole cottonseed meal.

Edgerton and Morris (1912) also conducted many feeding experiments with cottonseed and cottonseed meal. They fed sodium phosphate in large amounts and concluded that they had found "no evidence whatever to show that pyrophosphoric acid has anything to do with cottonseed-meal poisoning."

Rather (1912) also studied the phosphorus compounds of cottonseed meal and concluded that there was no evidence that the samples of cotton-seed meal examined contained either pyrophosphoric acid or metaphos-phoric acid. He also states (p. 16) that "the inorganic phosphorus (Forbes' method), in the samples of cottonseed meal examined was less than 5 per cent of the total phosphorus."

R. J. Anderson (1912, p. 5) isolated an inosite phosphoric acid very similar to phytic acid and made the following statement:

> The organic phosphoric acid of cottonseed meal gives all the reactions previously attributed to the presence of pyro- and meta-phosphoric acids. But the question whether or not it is also the toxic principle in cottonseed meal remains unanswered. Preliminary experiments carried out with the acid obtained from the purified barium salt on rabbits are not conclusive. Given in 0.5 and 1 gram doses, both the free acid and its potassium salt produced strong symptoms of distress, but after a few hours the animal regained their normal appearance. Larger doses passed through the bowel in a very short time and no definite symptoms developed.
>
> It is difficult to determine just what caused the toxicity of the preparations which were used in the experiments described by Crawford. It is evident that very impure substances were fed.

Since inosite phosphoric acids occur in numerous feeding stuffs other than cottonseed meal—e. g., wheat bran, corn, oats, barley—and since no suspicion of toxicity has occurred in these substances it seems highly improbable that the phosphoric acids in cottonseed meal have any significant action as toxic agents.

METHODS FOR REMOVING OR DIMINISHING THE TOXICITY OF COTTONSEED

Three methods have been proposed at the North Carolina Experiment Station and have been found effective for diminishing the toxicity of cottonseed kernels or cottonseed meal:

(1) Extraction of the kernels with ether (feed 316) or with ether and with alcohol (feed 331). By these methods gossypol is reduced to such a small amount that the residue is only slightly toxic (feed 316) or is nontoxic (feed 331).

(2) Treatment of the meal with an alcoholic solution of an alkali (Withers and Ray, 1913b). This treatment affords conditions for rapid oxidation, and oxidized gossypol has been found by us to be nontoxic (feed 338).

(3) Treatment of the meal with iron salts (Withers and Brewster, 1913) and Withers (1913). Treatment with iron salts is accompanied by some chemical action, as shown by the pronounced change in the color of the meal. The favorable physiological changes may be due to oxidation of the gossypol or to the formation of a more difficultly soluble compound. The oxidation may be due to the stimulating action of iron upon the oxidases of the animal body or to the direct action which ferric salts exert upon phenolic bodies. Ferrous sulphate forms an insoluble lake with gossypol. We have not yet studied it, but as Marchlewski (1899) found the lead salt so stable that it was not decomposed by hydrogen sulphid nor sulphuric acid, it is likely that the iron lake is very stable also.

The seed tissue surrounding the cells probably prevents the free action of reagents which would extract gossypol or render it physiologically inert. This constitutes the principal difficulty that must be overcome by the oil miller or stock feeder in rendering cottonseed meal nontoxic.

SUMMARY

(1) Gossypol, first isolated by Marchlewski from cottonseed oil and considered by him a prospective dyestuff, was extracted by us from cottonseed kernels and found to possess toxic properties.

(2) Cottonseed kernels were used as the initial material instead of cottonseed meal, because they yield gossypol more readily to solvents and are toxic to about the same extent.

(3) Ethyl ether was used as the solvent, the kernels having been extracted with gasoline to remove most of the oil. Evaporation of the ether leaves a crude product which we have designated "gossypol extract." A purer product, "precipitated gossypol," was obtained from the ethereal solution by the addition of gasoline, and a crystalline product, "gossypol 'acetate,'" by precipitation by acetic acid.

(4) Gossypol was fatal to rabbits when administered intraperitoneally in the form of gossypol extract or crystalline gossypol acetate, either when fed in one large dose in the form of gossypol extract or when fed in small daily doses in the form of gossypol extract, precipitated gossypol, or gossypol "acetate."

(5) Gossypol forms an oxidation product which is nontoxic.

(6) Cottonseed kernels are rendered less toxic by the partial extraction of gossypol and nontoxic by a more nearly complete extraction of it.

(7) Methods for rendering cottonseed kernels nontoxic depend upon extracting the gossypol or changing it to physiologically inert forms by oxidation or by precipitation.

(8) The smallest amount of gossypol administered intraperitoneally by us and found fatal to rabbits was 0.24 gm. of crystalline gossypol acetate per kilo of live weight.

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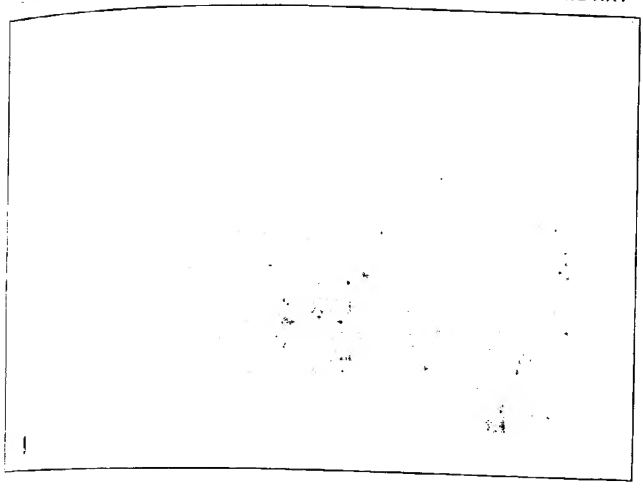
PLATE XXV

Gossypol glands of the cottonseed:

Fig. 1.—Lengthwise sections of cottonseed kernels, showing glands, folded cotyledons, and hypocotyl. ×8.

Fig. 2.—Cross sections of five widely different varieties of cottonseed kernels: *a*, Russell Big Boll; *b*, Willet's Red Leaf; *c*, Piedmont Long-Staple; *d*, Allen's Early; *e*, Wine Sap. ×8.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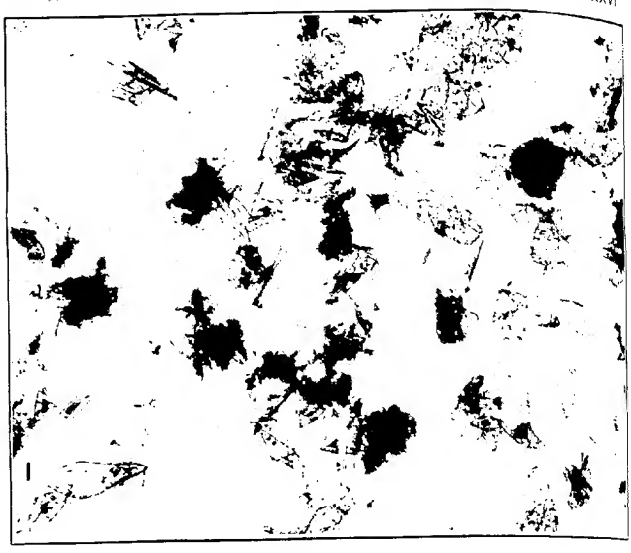
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PLATE XXVI

Fig. 1.—Crystals of gossypol "acetate" from alcohol and 50 per cent acetic acid. ×25.
Fig. 2.—Crystals of gossypol from acetone. ×25.

TWO NEW HOSTS FOR PERIDERMIUM PYRIFORME

By GEORGE GRANT HEDGCOCK, *Pathologist*, and WILLIAM H. LONG, *Forest Pathologist*, *Investigations in Forest Pathology, Bureau of Plant Industry*

Peridermium pyriforme Peck, which is the æcial form of *Cronartium pyriforme* (Peck) Hedgc. and Long, was collected for the first time on *Pinus rigida* Mill. by the senior writer on June 16, 1915, near Essex Junction, Vt. (F. P. 17708).[1] This is the first collection which has been reported on this host. The senior writer had previously found the uredinial and telial forms in abundance in the same locality on *Comandra umbellata* (L.) Nutt. (F. P. 8655) on July 31, 1913. This find is important, since it may serve to clear up the mystery associated with the identity of the host in the case of the type specimen on *Pinus* spp.,[2] collected by Prof. J. B. Ellis (2040) in 1880, possibly near Newfield, N. J., Ellis not being certain as to the locality. Since the telial form was collected by Ellis (Ellis and Everhart, N. A. Fungi, No. 1082) near Newfield in 1879 and as *Pinus rigida* is the only native species of pine in this locality known to be attacked by the fungus, it is very probable that this species is the host of the type. In measurements and shape the spores of the writers' specimen agree with those of the type which the writers have examined at the herbarium of the State Museum at Albany, N. Y. The type specimen consists of a young pine twig whose bark closely resembles in color and markings that of *Pinus rigida*.

Mr. Roy G. Pierce, of this office, collected a number of specimens of *Peridermium pyriforme* on *Pinus divaricata* (Ait.) Du Mont de Cours (Pl. XXVII, fig. 1) in several localities near Cass Lake, Minn., during the month of June, 1915 (F. P. 18044, 18046, 18047, 18058, 18060, 18072, and 18076). So far as the writers know, only one specimen of the fungus has hitherto been reported on *Pinus divaricata*, and that was found by Mr. J. J. Davis in Douglas County, Wis. Mr. Pierce reported that the fungus was common where he collected it, and it is probably common also in other localities. He also found the uredinial form, *Cronartium pyriforme*, on July 11, 1915, on *Comandra umbellata* in the same locality as one of his previous collections of the æcial form.

The junior writer also has a specimen of this rust (F. P. 19440) on *Pinus divaricata* collected at Roscommon, Mich., by State Forester Marcus Schaaf. This specimen was sent in with *Peridermium cerebrum*, which on this host produces typical globular swellings, while *Peridermium pyriforme* causes the typical fusiform swellings. *Peridermium pyriforme*, however, does not always produce fusiform swellings, since the junior writer has recently received a specimen (F. P. 19437) on a 4-year-old

[1] "F. P."=Forest-Pathology Investigations number.

[2] Hedgcock, G. G., and Long, W. H. A disease of pines caused by Cronartium pyriforme. U. S. Dept. Agr. Bul. 247, p. 7. 1915.

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transplant of *Pinus (murrayana) contorta* Loud., collected at Roscommon, Mich., by Mr. Schaaf, which produced a globoid gall (Pl. XXVII, fig. 2) extending nearly around the attacked stem. This gall was 6 cm. in circumference and 2 cm. in diameter. Both above and below the gall were irregular lesions caused by *Peridermium comptoniae* (Arthur) Orton and Adams. The gall resembled so closely the swelling produced by *Peridermium cerebrum* that the junior writer thought it was this species until he examined it under the microscope, when he found the typical pyriform spores of *Peridermium pyriforme*.

In June, 1915, the junior writer received a fine specimen of *Peridermium pyriforme* (F. P. 19429) on *Pinus arizonica* Engelm., a 3- to 5-leaved pine (Pl. XXVII, fig. 3), collected by Ranger J. H. Woolsey in Jacobson's Canyon, Crook National Forest, Arizona. This is the first time this rust has been reported on this host. Many of the æcia of the specimen were very large and unusually prominent, owing to their marked extension beyond the bark. Some were over 2 cm. long and from 5 to 6 mm. in height. The galls were of the effused type and were from 40 to 50 cm. long. One of the branches attacked was about 2 inches in diameter where the lesions occurred. Its bark was very rough and exfoliated by the action of the fungus. The lesions had completely surrounded the two branches for a distance of from 20 to 30 cm., but had not yet killed them.

The writers have previously found *Peridermium pyriforme* only on pines having two to three needles in the leaf cluster,[2] and the occurrence of the fungus as now reported on *Pinus rigida* and *Pinus arizonica* is of interest, since it adds to the list of known hosts two pines of the group bearing three needles in a cluster. *Pinus rigida* has three needles and *Pinus arizonica* three to five needles.

It is now known that *Peridermium pyriforme* causes three forms of disease on pines; one with slight or no hypertrophy, common on *Pinus divaricata*, *Pinus pungens* Michx., and *Pinus ponderosa scopulorum* Engelm.; a second causing a fusiform or spindle-shaped swelling and found on *Pinus arizonica*, *Pinus (murrayana) contorta*, *Pinus divaricata*, *Pinus ponderosa* Laws., *Pinus ponderosa scopulorum* Engelm., and *Pinus rigida;* and a third form, causing the formation of globose galls (Pl. XXVII, fig. 2) now first reported on *Pinus (murrayana) contorta*.

Peridermium pyriforme, especially when weathered, superficially resembles *Peridermium comptoniae*, with which the senior writer found it associated near Essex Junction, Vt., where he found 1 specimen of the former and nearly 50 of the latter species. It is quite probable that this resemblance has frequently caused it to be overlooked by collectors wherever two species occur together and that a more careful search for *Peridermium pyriforme* will greatly extend the known range of the disease of pines caused by it. The spheroid galls of *Peridermium pyriforme* resemble very closely the spheroid galls of *Peridermium cerebrum* (Pl. XXVII, fig. 2); and unless the spores are examined, this form might be easily mistaken for the latter fungus.

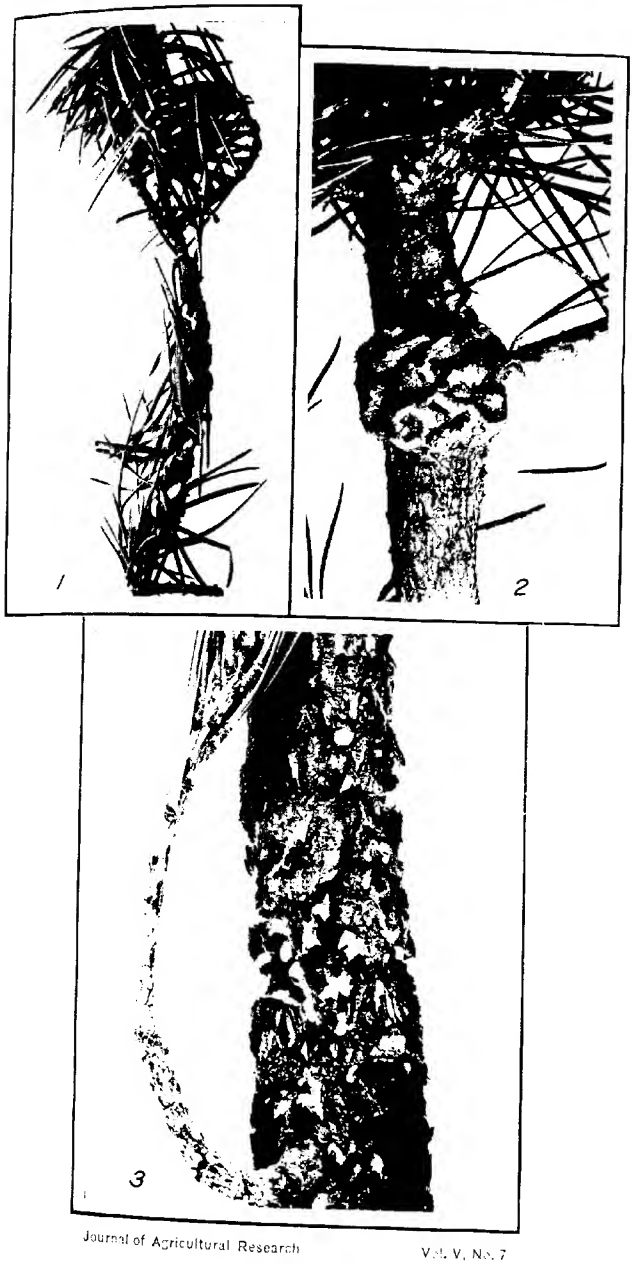
[2] Hedgcock, G. G., and Long, W. H. Op. cit.

PLATE XXVII

Fig. 1.—*Peridermium pyriforme* (F. P. 18044) on a trunk of *Pinus divaricata*, showing the form of the peridia before they are ruptured to allow the escape of the æciospores.

Fig. 2.—A globose gall with *Peridermium pyriforme* on a trunk of *Pinus contorta* (F. P. 19437), associated with two lesions of *Peridermium comptoniae*, one near the gall and the other 1 inch above it at the base of a branch.

Fig. 3.—*Peridermium pyriforme* (F. P. 19429) on a branch of *Pinus arizonica* showing unopened peridia. This branch was 1 inch in diameter and 10 years old.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PATHOGENICITY AND IDENTITY OF SCLEROTINIA LIBERTIANA AND SCLEROTINIA SMILACINA ON GINSENG

By J. ROSENBAUM,[1]

Mycologist, Cotton and Truck Disease Investigations, Bureau of Plant Industry

INTRODUCTION

For a number of years two species of Sclerotinia have been recognized as probable causes of the rotting of ginseng roots (*Panax quinquefolia*), but the pathogenicity and identity of these fungi have not been proved by by inoculation experiments.

The purpose of this paper is (1) to report inoculation experiments establishing the pathogenicity of these organisms, and (2) to detail the experimental data and considerations on which the conclusions as to the identity of the two pathogens are based.

WHITE-ROT OF GINSENG

The white-rot of ginseng was first reported by Whetzel (1907, p. 89).[2] Sclerotia were found, but the identity of the fungus was not determined. Subsequent workers, Rankin (1910), Osner (1911), and Whetzel and Rosenbaum (1912, p. 34–45) have attributed the disease to *Sclerotinia libertiana* Fuckel. These writers based their observations on the association of the sclerotia of the fungus with the host and the general resemblance of the organism on the host and in culture to the widespread *Sclerotinia libertiana*. No inoculation experiments have been reported.

PATHOGENICITY

During the spring of 1913 the fungus was isolated from diseased ginseng roots grown at Newtown, Pa., Mentor, Ohio, and Edenville, Mich. The isolations were made by washing the roots, immersing them for 10 minutes in a solution of mercuric chlorid (1 to 1,000), peeling back a portion of the external tissues, and transferring small bits of tissue from the inside of the root to poured plates of hard potato agar. Pure cultures were obtained in the majority of cases from the first planting. In addition to the cultures isolated from ginseng, inoculations on healthy ginseng

[1] The writer is indebted for many suggestions to Dr. Donald Reddick, of Cornell University, under whose direction this work was done.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 297.

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roots were also made with a culture of *Sclerotinia libertiana* obtained from lettuce from South Carolina. The procedure followed in the inoculations was as follows: Healthy ginseng plants with the tops still attached were selected and the soil carefully removed from one side of the root. By means of a flamed scalpel longitudinal cuts were made in the side of the root. These cuts were approximately one-fourth of an inch in length and about one-eighth in depth. A piece of agar containing mycelium from young cultures was inserted within these cuts and covered with soil. Check roots were treated in a similar manner.

During the summer inoculations were made as shown in Table I. The checks in every case remained healthy.

TABLE I.—*Results of the inoculation of ginseng with Sclerotinia libertiana from various sources*

Date.	Source of culture.	Number of roots inoculated.	Number of checks.	Percentage of infection.
July 14	*Sclerotinia libertiana* from South Carolina from lettuce	6	2	100
15	*Sclerotinia* sp. from Mentor, Ohio, from ginseng.	6	2	100
15	*Sclerotinia* sp. from Newtown, Pa., from ginseng.	8	4	100
15	*Sclerotinia* sp. from Edenville, Mich., from ginseng	6	2	83+
Aug. 1	*Sclerotinia* sp. from Mentor, Ohio, from ginseng.	4	1	100
1	*Sclerotinia libertiana* from South Carolina from lettuce	4	1	75

Plate XXVIII, figures 1 and 2, is reproduced from photographs of ginseng roots from two of the above series. Figure 1 shows a root inoculated with *Sclerotinia libertiana* isolated from lettuce. Figure 2 shows three roots (on the left) inoculated with a species of Sclerotinia isolated from ginseng.

Reisolations were made from the inoculations of July 15 and the fungus was again grown in pure culture. Inoculations made with the reisolated culture gave positive results.

Infection was evident in from three to seven days after inoculation. The root at the point of inoculation becomes soft and the rot spreads gradually in all directions, causing the entire root to beame soft and doughy. After the mycelium has penetrated throughout the tissues of the root, it forms tufts of cottony-white felt, in which large black sclerotia rapidly develop. Sclerotia on the outside of the root have in some cases developed within 10 days after the inoculations were made. When the inoculations are made near the crown of the root, the mycelium spreads to the stem, where it develops similar sclerotia on both the inside and the outside of the stem. The rapidity with which the disease progresses in the inoculted roots depends upon moisture conditions.

During a rainy period infection is evident within a much shorter time. All attempts to produce the disease without previously injuring the root gave negative results.

IDENTITY OF THE SPECIES

In order to further prove that the species of Sclerotinia from ginseng is identical with *Sclerotinia libertiana* Fuckel, a comparison was made with cultures from different sources. In addition to the four strains mentioned above, there was also used a pure culture isolated by Dr. Donald Reddick, of Cornell University, from celery. The comparison of the strains consisted in (1) growing the cultures on different media, both acid and alkaline; (2) production of apothecia, measurements of asci, ascospores, and a study of the manner of germination; (3) cross-inoculations on lettuce. These topics are briefly discussed in the following paragraphs.

GROWTH ON DIFFERENT MEDIA.—Cultures were made on potato agar, nutrient agar, bean plugs, ginseng stems, and Raulin's synthetic fluid. In the case of potato and nutrient agar both acid and alkaline media were used (±10.5 Fuller's scale). On all the media the various strains made a good growth, but no differences were visible.

PRODUCTION OF APOTHECIA, ETC.—In order to obtain apothecia from the various strains, the sclerotia produced in pure culture were placed on sterile moist sand in dome-shaped preparation dishes. The sclerotia were covered with a very thin layer of the sand, and the dishes were placed on a shelf in front of a window. The time required for these apothecia to develop varied greatly, the limits being from three weeks to three months. The size of the apothecia likewise varied even in the case of sclerotia from the same strain and produced in the same test tube. However, the apothecia were alike in general appearance in all the strains. Plate XXVIII, figure 3, shows apothecia from the celery strain, and Plate XXVIII, figure 4, shows the same from the ginseng strain. A large number of measurements made of asci, paraphyses, and ascospores showed no marked variations, and agreed with the description of *Sclerotinia libertiana* Fuckel as given in Saccardo. In figure 1, *A*, is shown a camera-lucida drawing of asci, ascospores, and paraphyses from a fresh preparation of the Mentor strain.

Crushed pieces of apothecia were placed in drops of water in order to observe the ascospore germination. Within four hours after being placed in water the first signs of germination became visible. Figure 1, *B*, shows the ascospores within the asci, germinated by sending germ tubes directly through the walls of the ascus. No differences were noted in the germination of the spores from the different strains.

INOCULATIONS ON LETTUCE.—Mature lettuce plants were selected and inoculated with the various strains of the fungus. Inoculations were

made on injured and uninjured plants, which were then covered with bell jars for 4 days. At the end of 12 days most of the plants showed signs of rotting. Unlike the ginseng roots (Pl. XXVIII, figs. 1 and 2) previously discussed, infection occurred not only on the injured, but also on the uninjured plants.

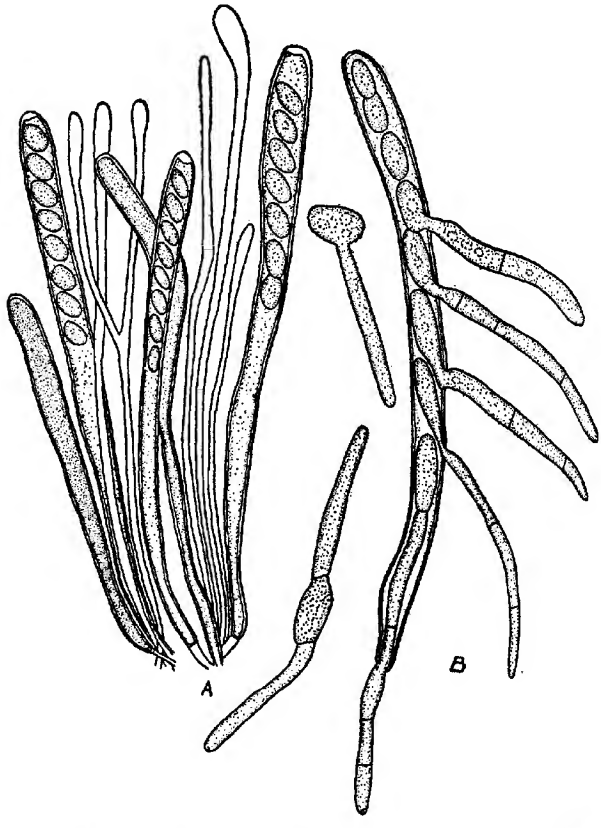

FIG. 1.—*Sclerotinia libertiana: A*, Camera-lucida drawing showing branched and unbranched paraphyses, asci, and ascospores; *B*, camera-lucida drawing showing methods of ascospore germination. Those within the asci germinate by sending germ tubes directly through the walls of the ascus.

BLACK-ROT OF GINSENG

Van Hook (1904, p. 181–182) first mentions a species of Sclerotinia as the cause of a black-rot of ginseng. Rankin (1912) reports the discovery of the apothecia and established a new specific name for the fungus. No inoculations were attempted, either on the ginseng roots or on other hosts known to be attacked by species of Sclerotinia closely allied to this one.

PATHOGENICITY

In the spring of 1912 the writer received a number of black-rotted roots from Wisconsin showing various stages of development of the disease. Isolations were made from these roots by making plantings from the inner tissues of the roots on poured plates of hard potato agar. The fungus was obtained in pure culture, where it produces a characteristic black growth.

Inoculations on healthy roots made at various times during the summer gave negative results, as would be expected from the nature of the fungus, since the disease always develops in beds during the winter. In October of the same year (1912) six roots were washed clean and inoculated by placing a piece of the agar pure culture in a small cut made in the tissues of the root. Three similar roots were injured and used as checks. All the roots were planted in soil which had never grown a crop of ginseng. The following March an examination of the roots showed the characteristic symptoms of the disease. Some were entirely black, while others were only partly blackened. The fungus was easily reisolated from these roots. Plate XXIX, figure 1, shows two inoculated roots, together with a check root. One of the inoculated roots is entirely black, while the second shows this black color only in part.

In October, 1913, inoculations were again made on ginseng roots. These roots were not injured, but the fungus was placed on the old stem scar. The next March the roots were black, as in the previous year. Reisolations were again made, and the fungus which was obtained produced the characteristic black growth.

IDENTITY OF THE SPECIES

The growth of the fungus in culture and the general behavior of this organism differed so greatly from the known species of Sclerotinia that it has always been an interesting question as to the source of the fungus which appeared in isolated gardens throughout the country. One plausible explanation is that the fungus, being associated with wild ginseng roots or with one of the common weeds, was brought in from the woods, as many growers make a practice of using leaf mold in preparing their beds. Since the fungus from the description resembled *Sclerotinia smilacina* Durand, it seemed advisable to determine whether the species of Sclerotinia on ginseng could produce a black-rot of the rhizome of *Smilacina* spp. and whether the two were also identical in other respects.

INOCULATIONS ON SPECIES OF SMILACINA.—In October, 1913, six rhizomes of *Smilacina racemosa* were inoculated with a pure culture of the black-rot fungus obtained from ginseng. The inoculations were made by slightly injuring the rhizome and inserting the mycelium of the fungus in the cut. Check plants were also injured. When examined the following March, the rhizomes showed the characteristic symptoms of black-rot

as exhibited by ginseng roots. The check plants remained healthy. Plate XXIX, figure 2, is a reproduction of a photograph of two of the inoculated and one check rhizome. Reisolations were made, and the fungus which was obtained resembled the original culture isolated from ginseng.

COMPARISON WITH TYPE SPECIMEN.—To determine further the relationship of the *Sclerotinia* sp. from ginseng to that on *Smilacina* spp., an examination was made of the type specimen of *Sclerotinia smilacina* Durand, deposited by Dr. Durand in the herbarium of the botany department of Cornell University. The specimens showed the black coloration as exhibited by the inoculated rhizomes of *Smilacina racemosa* as well as the ginseng roots.

Apothecia on ginseng are rare, and though attempts to produce them were made no success can be reported up to the present time. It is of interest, however, to compare the measurements as given in the original descriptions by Durand (1902, p. 462–463) and Rankin (1912) as shown in the following table:

Species.	Sclerotia.	Apothecia.	Asci.	Ascospores.
	Gm.	*Gm.*	μ	μ
Sclerotinia smilacina..	0.1 by 0.2 to 2.	0.75 to 3..	120 to 140 by 6 to 8.	12 to 15 by 4 to 5.
Sclerotinia panacis....	0.3 to 1.....	1.5 to 2.5..	125 to 137.5 by 6.4 to 6.5.	11.7 to 16 by 4.8 to 7.5.

Measurements made by the writer from the type material of these species have shown that the asci and ascospores are not to be distinguished either in form or size and agree with the measurements given above.

CONCLUSIONS

1. (A) The pathogenicity of *Sclerotinia* sp. causing the white-rot of ginseng has been established. (B) This species of Sclerotinia is identical with the *Sclerotinia libertiana* Fuckel occurring on lettuce, celery, and a number af other hosts.

2. (A) The pathogenicity of *Sclerotinia* sp. causing the black-rot of ginseng has been established. (B) A consideration of the following facts indicates that *Sclerotinia panacis* Rankin is identical with *Sclerotinia smilacina* Durand: (a) Inoculations with a species of Sclerotinia from ginseng on *Smilacina racemosa* gave positive results. (b) Measurements of asci and spores made by the writer from the type material of both species agree. There is a close agreement in all distinguishing characters, as given in the original description of the two species. (c) The lesions produced by the inoculations are similar on the two hosts and identical with those on diseased plants as they occur naturally.

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PLATE XXVIII

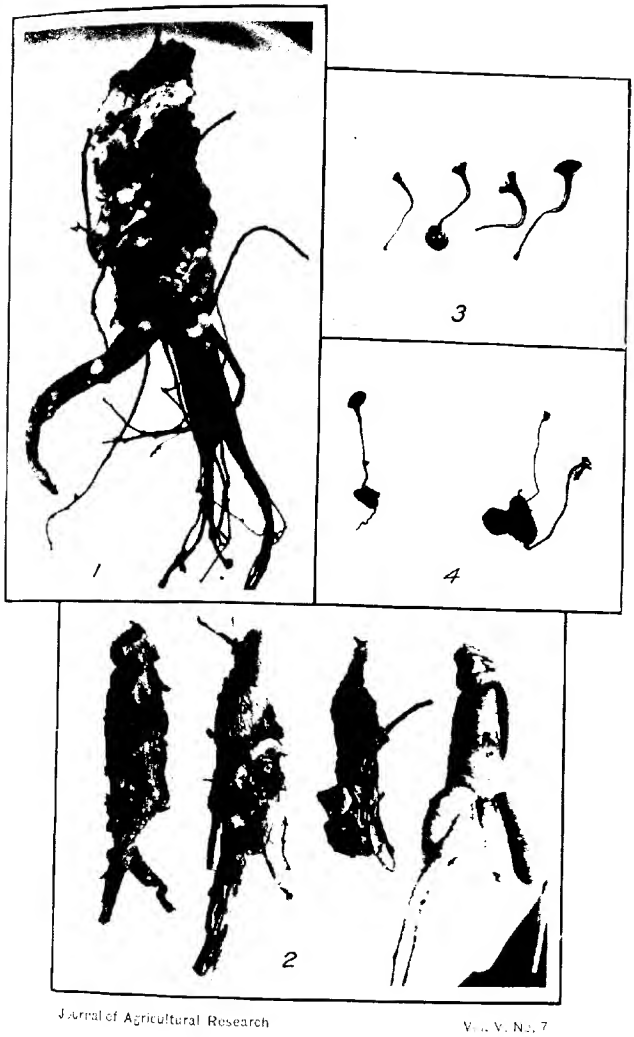
Sclerotinia libertiana:

Fig. 1.—Root inoculated with *Sclerotinia libertiana* from lettuce. Note the white mycelial felt and the production of sclerotia.

Fig. 2.—Three roots (on left) inoculated with *Sclerotinia* sp. from ginseng. Healthy check root (on right).

Fig. 3.—Apothecia from sclerotia from celery strain.

Fig. 4.—Apothecia from sclerotia from ginseng strain.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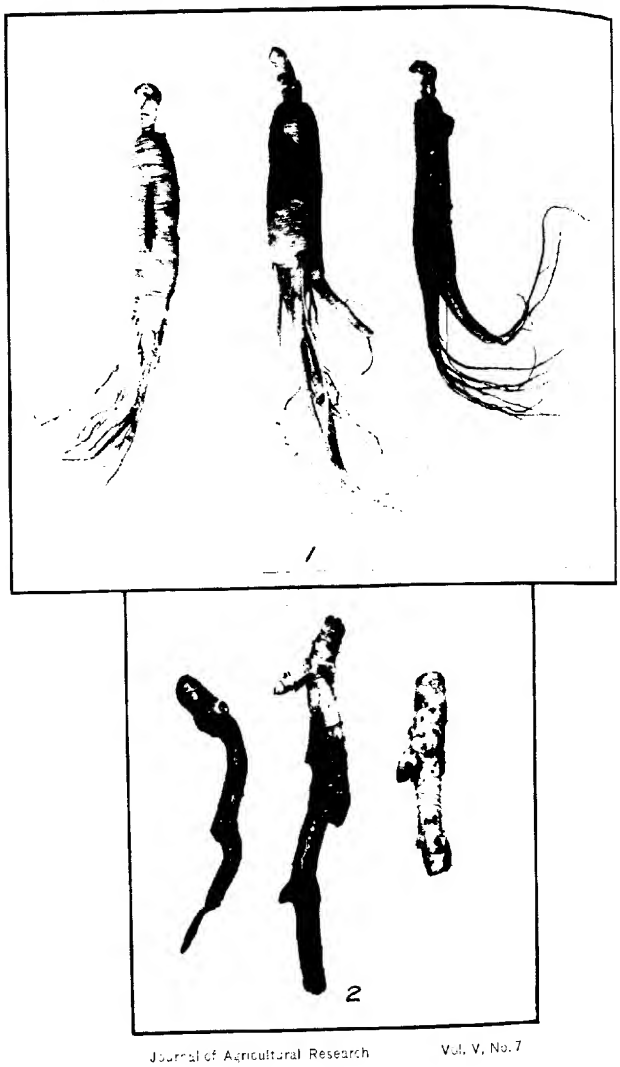

PLATE XXIX

Sclerotinia smilacina:

Fig. 1.—Ginseng roots showing the characteristic black color from artificial inoculation. The root on the left is the check.

Fig. 2.—Rhizomes of *Smilacina racemosa* inoculated with a species of Sclerotinia isolated from ginseng. The rhizome on the right is the check.

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